Capacity for Infectious HIV-1 Virion Capture Differs by Envelope Antibody Specificity

Pinghuang Liu,^{a,c} LaTonya D. Williams,^{a,c} Xiaoying Shen,^{a,c} Mattia Bonsignori,^{a,c} Nathan A. Vandergrift,^{a,c} R. Glenn Overman,^{a,c} M. Anthony Moody,^{a,f} Hua-Xin Liao,^{a,c} Daniel J. Stieh,^g Kerrie L. McCotter,ⁱ Audrey L. French,^{i,j} Thomas J. Hope,^g Robin Shattock,^h Barton F. Haynes,^{a,c,d} Georgia D. Tomaras^{a,b,d,e}

Duke Human Vaccine Institute^a and Departments of Surgery,^b Medicine,^c Immunology,^d Molecular Genetics and Microbiology,^e and Pediatrics,^f Duke University, Durham, North Carolina, USA; Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA^g; Mucosal Infection & Immunity Group, Section of Infectious Diseases, Imperial College London, St. Mary's Campus, London, United Kingdom^h; CORE Center of Cook County Health and Hospitals System, Chicago, Illinois, USAⁱ; Rush University Medical Center, Chicago, Illinois, USA^j

Antibody capacity to recognize infectious virus is a prerequisite of many antiviral functions. We determined the infectious virion capture index (IVCI) of different antibody specificities. Whereas broadly neutralizing antibodies (bNAbs), except for an MPER bNAb, selectively captured infectious virions, non-bNAbs and mucosal human immunodeficiency virus type 1 (HIV-1)-positive IgG captured subsets of both infectious and noninfectious virions. Infectious virion capture was additive with a mixture of antibodies, providing proof of concept for vaccine-induced antibodies that together have improved capacity to recognize infectious virions.

Mounting evidence indicates that human immunodeficiency virus type 1 (HIV-1) binding antibodies, in addition to broadly neutralizing antibodies (bNAbs), might be able to provide some protection against HIV-1 transmission *in vivo*. Potentially protective antibody functions include direct neutralization, Fc-mediated antibody function (e.g., antibody-dependent cellular cytotoxicity [ADCC], antibody-dependent cell-mediated virus inhibition [ADCVI]), transcytosis inhibition, viral aggregation, and inhibition in mucus (1–4). Antibodies mediate effector function either by recognizing infected cells or infectious HIV-1 virions. Thus, the ability of antibodies to recognize and bind avidly to infectious virions is a prerequisite of numerous potentially protective antibody functions. In simian-human immunodeficiency virus (SHIV) challenge studies, the capacity of passively infused monoclonal antibodies (MAbs) to mediate virion capture was implicated in the protection against mucosal transmission of SHIV in macaques (5, 6). We previously reported that infectious virion capture occupies unique immunological space among antibody functions (7). *In vitro*-prepared virus stocks are a heterogeneous population of quasispecies composed of infectious virions as well as defective noninfectious virus particles. Virus stocks differ in HIV-1 sequences, culture conditions used for their generation, and target cells used for infectivity assays (8, 9). It has been suggested that it requires on the order of 8 fusion events to lead to an integrated provirus (10). Plasma virions have a mean half-life of approximately 6 h (11) and can shed the gp120 component of the envelope, leading to virions defective for infectivity. An ideal antibody to induce by vaccination is one that can selectively and avidly capture all infectious virions. Thus, the ability of antibody to selectively capture infectious virions may be an important trait to be induced by a vaccine. In this study, we investigated a panel of MAbs and purified mucosal IgG antibodies of different specificities to directly compare breadth of virion capture and to determine what fractions of infectious virions they can recognize.

We established an infectious virion capture assay (IVCA) to distinguish selective capture of infectious virions compared to noninfectious virions (7, 12). We (7) and others (8, 13) reported

that HIV-1 virion capture measurements represent unique immunological space from standard neutralizing antibody assays. The IVCA method utilizes a protein G column-based capture of immune complexes with two readouts for quantitation of total virus particles (reverse transcription [RT]-PCR) or the infectious virions (TZM-bl infectivity assay) (7, 12). We characterized 12 MAbs that target different HIV-1 Env epitopes, including Env gp120 V1/V2, CD4 binding site, glycan, gp120 conformational epitopes, Env gp41, and polyclonal purified HIV-1 IgG (HIVIG) for the ability to capture infectious virions with a panel of 11 HIV-1 strains, including subtypes B, A/E, and C (Fig. 1A).

Capacity and breadth of infectious virion capture depend on antibody specificity. The bNAbs 2G12, PG9, PG16, CH01, and CH31 selectively captured higher proportions of infectious virions (iVirions; measured by infectivity) compared to total virus particles (rVirions; measured by RNA copies) (Fig. 1A, colored squares), indicating that they selectively bind to iVirions compared to rVirions. We then studied a set of antibodies that selectively captured infectious virions and compared them to a set of Abs that did not selectively capture infectious virions. The percentage of iVirion capture compared to rVirion capture across multiple HIV-1 strains is shown in Fig. 1. MAb 2G12 (targets a glycan configuration on the outer domain of gp120 [14]), MAbs PG9, PG16, and CH01 (target Env V1/V2 and V3 region [15, 16]), MAb 697-30D (targets Env gp120 V2 region [17]), VRC01-like MAb CH31 (targets CD4 binding site [18]), the nonneutralizing/weakly neutralizing MAbs 7B2 and F240 (target nonneutralizing

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Address correspondence to Georgia D. Tomaras, gdt@duke.edu.

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FIG 1 Distinction of infectious virion capture (iVirion) compared to total virus particle capture (rVirion). (A) iVirion capture and rVirion capture were measured for 12 HIV-1 IgG MAbs and polyclonal HIV purified IgG (HIVIG) from plasma against different HIV-1 strains (each spot represents an HIV-1 strain). (B) Individual plots for six MAbs are shown. Briefly, IgG was mixed with HIV-1 virions containing approximately 2×10^7 RNA copies/ml of HIV-1 stock at a final concentration of $10 \mu\text{g/ml}$ ($300\text{-}\mu\text{l}$ vol), and the *in vitro*-prepared Ab-virion immune complexes (IC) were passed through a protein G column. The infectivity of the flowthrough was measured by a TZM-bl infection assay. The virus particles in the flowthrough and the column captured fraction were measured by HIV-1 *gag* real-time RT-PCR. The percentages of captured iVirion or rVirion were calculated independently with different denominators as follows: $\text{iVirion} = [(100 - \text{the flowthrough infectivity}) / \text{virus-only, no-Ab infectivity}] \times 100\%$ and $\text{rVirion} = [\text{captured viral RNA copies} / (\text{captured viral RNA} + \text{flowthrough viral RNA})] \times 100\%$ (7). The cutoffs for iVirion capture and rVirion capture were 15% and 10%, respectively, based on the mean of negative controls $\pm$ standard errors of the means (SEM). The percentage of iVirion is plotted against the percentage of virus particles (rVirion) captured by MAbs (at a concentration of $10 \mu\text{g/ml}$). Each symbol represents an Ab-virus capture measurement. Three to 11 viruses were tested for each MAb (clade B [circles], lab-adapted NL4-3, MN, and BaL, transmitted/founder [T/F] (30) CH077, CH058, CH040, and WITO; CRF01_AE [squares], 92TH023 and CM244; and clade C [triangle], CAP45, T/F CH185.c).

gp41 immunodominant epitope [5, 19]), bNAbs 10E8 (targets the MPER [21]) and polyclonal HIVIG (targets a mixture of epitope specificities) were analyzed for both breadth of virion capture and relative level of iVirion compared to rVirion capture. The bNAbs PG9, at $10 \mu\text{g/ml}$, captured 100% of CRF01_A/E infectious virions AE.92TH023 and AE.CM244, as well as subtype B MN virus (based on infectivity in the flowthrough from the column). In contrast, only 53% to 71% of rVirions were captured for these viruses, as measured by viral RNA (Fig. 1B). The difference in the level of infectious compared to total particle capture indicates that

PG9 selectively binds to functional virions. Similar results were obtained with other bNAbs, such as 2G12, CH01, and CH31 MAbs (Fig. 1A and B). Notably, the ability of these MAbs to selectively capture iVirions was observed across different HIV-1 strains, indicating breadth of virion capture. However, infectious virion capture can differ from neutralization potency. In particular, some MAb-virus combinations with more moderate or lower neutralizing titers have a broader range of percentages of infectious virion capture (data not shown).

V1/V2 MAbs CH58 and CH59 (20), nonneutralizing MAb

FIG 2 Infectious virus capture index (IVCI) quantifies preferential capture of infectious compared to noninfectious virions. (A) The IVCI of selective infectious virion capture for 8 MABs and HIVIG across 11 viruses are shown for those that are virus capture positive. $IVCI = \text{Ab} + \text{virus flowthrough (RNA copies/RLU)} / \text{virus-only flowthrough (RNA copies/RLU)}$. An index of >1.0 indicates preference for selective virus, and an index of 1.0 indicates no preference in selection of infectious compared to noninfectious virions. The red dotted line indicates no infectious virus selection ($IVCI = 1$). Each symbol represents a distinct HIV-1 strain. The black lines are the mean IVCI across viruses for each MAB. (B) MAB 7B2, but not bNAb CH31, captures noninfectious virions. Infectious CM244 in the virus stock was depleted by PG9, and the noninfectious fraction of virus was used to assay for virus capture (as measured by viral RNA). MAB 7B2 captured $65,882 \pm 11,761$ absolute copies of viral RNA in the capture fraction for HIV-1 infectious virion-depleted CM244 stock (after virus only subtraction [Virus no-Ab]) and captured $19.5\% \pm 2.7\%$ total virion particles ($n = 2$), respectively. The results are the means of Ab-mediated rVirus capture percentages (with virus-only background subtracted) from two independent experiments. The error bar represents the SEM. Virus no-Ab, the virus-only negative control.

7B2, bNAb 10E8, and HIVIG captured similar proportions of iVirions and rVirions (Fig. 1B), although these antibodies were able to capture some fraction of infectious virions. Notably, the broadly neutralizing 10E8 MAB (21) that targets the membrane-proximal external region (MPER) in gp41 had lower levels of virion capture (up to $\sim 40\%$ on the virus strains tested here) than other broadly neutralizing antibodies ($>80\%$). These data are consistent with data by Chen et al. (22) that the 10E8 MAB does not recognize the untriggered form of Env. Taken together, these data indicate that the selection of infectious virion capture is related to virion antigenicity and antibody epitope specificity (9, 23).

IVCI measures Ab capacity for recognizing infectious and noninfectious virus particles. In order to quantify the selective ability of MABs to capture iVirions, we devised an infectious virion capture index (IVCI), representing the fold change of the ratio of iVirions relative to the rVirions calculated in the assay. The ratio was then normalized to the ratio of rVirion to iVirion in the flowthrough of the virus only (without antibody) to account for variation in the fractions of infectious virions across different virus stocks as calculated here: $IVCI = (\text{RNA copies of flowthrough/RLU of flowthrough}) / (\text{RNA copies of the virus-only flowthrough/RLU of the virus-only flowthrough})$, where RLU is relative light units. As expected, the IVCI of the negative-control MAB (palivizumab) (24, 25) was 0.96 ± 0.06 (range, 0.63 to 1.21) across 11 HIV-1 strains comprising HIV-1 subtypes B, A/E, and C; the IVCI of HIV-1-negative IgG (Sigma) (Hu-IgG) was 1.00 ± 0.04 (range, 0.71 to 1.11) across 10 different HIV-1 strains. The IVCI for the V2 MAB CH58 and gp41 MAB 7B2 were 0.75 ± 0.05 and 0.80 ± 0.07 (Fig. 2A). However, these MABs were also capable of capturing a fraction of infectious virions from multiple HIV-1 strains (Fig. 1). These results indicate that some antibodies are capable of capturing both infectious virions and noninfectious virus particles, with a preference toward capturing noninfectious virus particles (compared to iVirions).

To further confirm that MAB 7B2 was capable of capturing noninfectious virions, we performed a virus capture assay utilizing purified noninfectious virions that were enriched by depletion of the infectious virion fraction with PG9 at $20 \mu\text{g/ml}$. The depletion efficiency was confirmed by TZM-bl infection. After sub-

tracting the virus-only control, we calculated that MAB 7B2 captured more than 20% noninfectious rVirions (Fig. 2B). This is consistent with the notion that there are coexisting functional Env gp120/gp41 trimers, nonfunctional gp120/gp41 monomers, and gp120-depleted gp41 stumps on the HIV-1 particle surface (26). Interestingly, IVCI of HIVIG was 0.7 ± 0.1 ($P < 0.01$ compared to virus only; Mann-Whitney U test), although polyclonal HIVIG neutralizes a range of HIV-1 isolates (27). Thus, polyclonal HIVIG has a large fraction of antibodies that selectively capture noninfectious virions but also has a smaller fraction of antibodies that can bind avidly to infectious virions that mediate HIV-1 neutralization. In contrast, the IVCI of bNAbs 2G12, PG9/PG16, CH01, and CH31 were significantly higher than that of MAB 7B2 (all, >1.0 ; $P < 0.05$, Mann-Whitney test). IVCI ranged from 1.55 to 3.3 (mean, 2.4 ± 0.5) for CH01 (captured 3 out of 4 HIV-1 strains), 1.8 to 27.2 (mean, 11.6 ± 4.7) for PG9 (captured 5 out of 6 HIV-1 strains), 4.9 to 16.5 (mean, 11.4 ± 2.9) for PG16 (captured 4 tested strains), 1.27 to 46.2 (mean, 15.9 ± 7.9) for 2G12 (captured 7 out of 10 strains), and 3.8 to 36.8 (mean, 13.8 ± 7.7) for CH31 (captured 4 tested strains). MAB 2G12 did not capture HIV subtype B T/F CH040, subtype C CH185.C, or subtype A/E AE.92TH023 and exhibited an index of 0.9, 1.0, or 0.9, respectively. The markedly higher IVCI of MAB CH31 than that of MAB 7B2 is consistent with the finding that CH31 did not capture noninfectious CM244 virus particles (Fig. 2B). These results demonstrated that IVCI can quantitatively represent the ability of antibodies to selectively capture infectious virions.

Ab concentration dependence of HIV-1 virion capture. Next, to determine how Ab concentration influences the capacity to capture infectious virions, we titrated CH31 and PG9 MABs and the nonneutralizing MAB 7B2. We tested antibody concentrations ranging from $20 \mu\text{g/ml}$ to $7.9 \times 10^{-2} \mu\text{g/ml}$ for MABs PG9 and CH31 and from $20 \mu\text{g/ml}$ to $1.2 \times 10^{-3} \mu\text{g/ml}$ for 7B2 (4-fold dilution). MABs CH31 and PG9 captured virus in a dose-dependent manner (Fig. 3A). MAB CH31 captured 100% of infectious NL4-3 at a high concentration of $20 \mu\text{g/ml}$ but captured only 31% of total viral particles in this virion preparation. However, this level of virion capture rapidly dropped to background levels at $0.31 \mu\text{g/ml}$ for MAB CH31. The iVirion capture 50% inhibitory

FIG 3 Differential dependence of Ab concentration on virion capture by selective MABs and the nonselective MAB. Increasing concentrations of MABs that select for infectious virions (MABs CH31 and PG9) (A) or do not select for infectious virions (MAB 7B2) (B) were used to capture HIV-1 NL4-3 virions. The slashed green or black lines represent the cutoff values for the capture of iVirions or rVirions, respectively. Green symbols are infectious virion capture (iVirion); black symbols are total virion capture (rVirion). (C) The IVCI at each MAB dilution with detectable virus capture. The lines represent means $\pm$ SEM. MAB 7B2 IVCI was <1.0 across all dilutions. Similar results were seen with HIV-1 subtype A/E CM244 virus.

concentration (IC_{50}) for MAB CH31 was $1.08 \mu\text{g/ml}$, and the IVCI of MAB CH31 ranged from 15.9 to 1.37. Although, bNABs PG9 and CH31 showed similar capacity to capture iVirions and rVirions at $20 \mu\text{g/ml}$, at $0.08 \mu\text{g/ml}$, PG9 captured more than 50% of infectious NL4-3, whereas CH31 did not. Thus, the PG9 MAB displayed higher levels of virion capture at lower concentrations with a calculated iVirion capture IC_{50} of $0.27 \mu\text{g/ml}$ compared to $1.08 \mu\text{g/ml}$ for MAB CH31. The PG9 IVCI ranged from 8.2 to 2.0 at Ab concentrations ranging from $20 \mu\text{g/ml}$ to $7.9 \times 10^{-2} \mu\text{g/ml}$ (Fig. 3C). In contrast, the nonselective MAB 7B2 captured a similar fraction of both infectious NL4-3 and rVirions across Ab concentrations ranging from $20 \mu\text{g/ml}$ to $4.9 \times 10^{-3} \mu\text{g/ml}$ (Fig. 3B). Unlike CH31 and PG9, MAB 7B2 did not capture all infectious NL4-3 at the highest concentrations tested ($20 \mu\text{g/ml}$). However, MAB 7B2 captured approximately 20% of both iVirions and rVirions even at low concentrations ($4.9 \times 10^{-3} \mu\text{g/ml}$). The measured index represents the ratio change of infectious virions to total virion particles in the flowthrough fraction relative to the virus-only control after passing the protein G column. The amount of captured infectious virions can decrease with declining antibody concentration since fewer infectious particles overall can be captured at lower Ab concentrations. However, when at the same antibody concentration, the antibody with highest affinity for the most functional forms of Env should exhibit the highest index.

These data indicate that the infectious population of NL4-3 is

comprised of multiple subpopulations of infectious virions, one of which has no accessible immunodominant epitope targeted by the 7B2 MAB. The IVCI of MAB 7B2 was less than 1.0 across the entire range of antibody concentrations (range, 0.56 to 0.99) (Fig. 3C). Additional studies conducted with the CRF01_A/E primary isolate AE.CM244 showed similar results (data not shown). Taken together, these data indicate that there is a fraction of virions in the NL4-3 virus stock that have only functional Env trimers on the surface. Alternatively, there may be nonfunctional forms of envelope present, but the spacing of the different forms of Env on the surface of virion or other factors may influence the accessibility of some epitopes for Ab-mediated virion capture. Thus, an inherent feature of selective infectious virion capture is independent of antibody concentration and rather is dependent on the proportion of virions appropriately exposing the targeted epitope on the virions.

Mucosal HIV-1 envelope IgG captures infectious virions.

One potentially protective mechanism against HIV-1 acquisition is the presence of antibodies at mucosal surfaces that can recognize infectious virions. Thus, we examined genital mucosal IgG purified from HIV-1-infected women to determine whether mucosal IgGs could capture infectious virions and discriminate between infectious and noninfectious virions. Vaginal secretions were collected from consenting women with a Weck-Cel sponge from the ectocervical posterior fornix over 60 s. HIV-1 Env-specific IgG was detected in all three patients' mucosal samples, with

FIG 4 Mucosal HIV-1-specific polyclonal IgG from vaginal Weck-Cel sponge from HIV-1-positive women can capture a fraction of infectious HIV-1 virions. (A and B) Black bars represent the capture of iVirion, and the gray bars represent the capture of rVirion. The error bar is the SEM from 3 replicate wells from two independent experiments. (C) The percentage of captured iVirion was plotted against the percentage of captured rVirion for mucosal weck-purified IgG. PTID4, ■; PTID6, ▼; PTID7, ▲; HIVIG, ●; HIV-1 Bal, red symbols; and HIV-1 NL4-3, black symbols.

FIG 5 Infectious virus capture improves with addition of MAbs of different specificities. The mixture of V2 MAb CH58 and gp41 MAb 7B2 captured more virions compared to CH58 or 7B2 alone for HIV-1 subtype A/E 92Th023. (A) iVirion capture is shown in black and rVirion capture is shown in gray. (B) The median IVCI for mixture of V2 MAb CH58 and gp41 MAb 7B2 was higher than that of MAb 7B2 alone. The results are representative of four independent experiments. *, $P < 0.05$ by exact Wilcoxon test.

HIV-1 IgG concentrations ranging from 2.7 to 10.8 $\mu\text{g/ml}$, relative to the standard 2G12 MAb for binding to HIV-1 63521 clade B T/F Env gp120. We found that mucosal IgG from one patient (PTID4) captured infectious virus particles (NL4-3); however, two others (PTID6 and PTID7) captured only low levels of virus (Fig. 4A). Mucosal IgG from PTID4 also captured subtype B HIV-1 BaL, whereas PTID6 and PTID7 captured lower levels of BaL, similar to that seen for NL4-3 (Fig. 4B). Similar to HIVIG, mucosal IgG from vaginal wecks did not selectively capture infectious virions (Fig. 4C). The mean IVCI of the three mucosal IgG samples was 0.3 ± 0.2 for NL4-3 and 0.5 ± 0.01 for BaL, similar to HIVIG (0.46 for NL4-3 and 0.49 for BaL). These results show that mucosal HIV-1-specific IgG from HIV-1-infected women reproducibly captured a fraction of infectious virions.

Combination of HIV-1 Env MAbs increases infectious virion capture. To test whether MAbs targeting different HIV-1 epitopes could increase the amount of infectious virions captured, we measured the ability of the V1/V2 MAb CH58 and gp41 MAb 7B2 to capture HIV-1 AE.92TH023 virions. We found that MAbs CH58 and 7B2 combined at a 1:1 ratio significantly captured more virions than each alone, as measured by both iVirions and rVirions ($P < 0.05$, MAb CH58 compared to MAb CH58 and 7B2 mix; $P < 0.05$, MAb 7B2 compared to MAb CH58 and 7B2 mix by exact Wilcoxon test) (Fig. 5A). The IVCI of CH58 and 7B2 MAbs together also significantly increased compared to that of each MAb alone (Fig. 5B), indicating improved selection of infectious virion capture when mixed together.

In summary, we devised an HIV-1 virion capture analysis that distinguishes between selective capture of infectious and noninfectious virions. This selection is dependent on antibody epitope specificity and the HIV-1 strain. Some antibody specificities, although not selective for only infectious virus capture, can still capture a fraction of infectious virions at very low Ab concentrations. Antibodies that are not broadly neutralizing likely have limited capacity to recognize native trimers (28); however, they may recognize nonfunctional forms of envelope (26, 29) also present on some infectious virions. As proof of concept for a vaccine strategy, we demonstrated that a combination of two MAbs of different specificity could increase the overall fraction of infectious virus

that is captured compared to a single specificity alone. HIV-1 infection can induce mucosal IgG with limited capacity for infectious virion capture. Additional studies are needed to determine whether a more diverse cocktail of multiple antibody specificities can further improve upon capture of infectious virions at the mucosal portal of entry. A polyclonal antibody response, including antibodies that can capture infectious virions, may be part of an effective HIV-1 vaccine strategy.

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